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*Andrei D. Mirzabekov*  
Professor, Director

ENGELHARDT INSTITUTE of MOLECULAR BIOLOGY  
RUSSIAN ACADEMY OF SCIENCES

Attention: Nick Sutcliffe  
Fax: +44 20 7240-9339

### Declaration

My extensive studies into the properties of nucleic acid hybridization, conducted over many years, have demonstrate that there are fundamental differences between the hybridization kinetics, chemistry, and capacity of solid surface 2D reaction surfaces compared to 3D supports comprised of gel matrices. Specifically, whereas a 2D surface is planar without significant liquid volume, a 3D support comprises ~95% liquid volume. The fact is that 3D supports have far greater typical molecular binding capacity, yield far greater hybridization signal strengths, and allow hybridization to proceed in a manner that is far more similar to liquid-based reactions rather than as would occur during 2D planar surface hybridizations.

The above characteristic differences between 2D surfaces and 3D supports are now widely known and accepted, and have been emphasized in several of my own peer reviewed publications. For example;

A) In the article 'Livshits MA, Florentiev VL, Mirzabekov AD. Dissociation of duplexes formed by hybridization of DNA with gel-immobilized oligonucleotides. J Biomol Struct Dyn, 1994, 11:783-795' we specified that our work employs a

"...matrix of oligonucleotides immobilized within polyacrylamide gel. The particular feature of this matrix is that the apparent thermostability [hybridization] of the duplexes depends on the concentration of gel-immobilized oligonucleotides. This dependence is specific for oligonucleotides immobilized in the gel volume (3-D-immobilization) rather than on a flat surface of a filter or glass (2-D-immobilization).

B) In 'Kolchinsky A, Mirzabekov A. Analysis of SNPs and other genomic variations using gel-based chips. Human Mutation, 2002, 19:343-60' we pointed out that

"Tethering molecules within a three-dimensional gel significantly, 102 to 103 times, increases the immobilization capacity as compared with equivalent glass [2D] surface",

and

"Interactions of macromolecules with gel-immobilized compounds occurs in the surrounding homogeneous liquid, and the physics of these processes is more similar to water solution than heterophase interactions with [2D] glass-immobilized compounds."

In total, I and others have now published many tens of papers on this subject over the last decade or more, with essentially all findings being in support of the above summarized observations. The conclusion is clear: hybridization data obtained from studies on 3D supports cannot be extrapolated to 2D surfaces.

Prof. A.D. Mirzabekov

30.01.03



Vavilova str. 23, Moscow 119991, Russia

Phones: +7 (095) 135-2311, 135-0559; Fax: +7 (095) 135-1405; E-mail: amir@eimb.ru